Biosystems Engineering (2003) **84** (1), 1–12
doi:10.1016/S1537-5110(02)00236-2
AE—Automation and Emerging Technologies

REVIEW PAPER

Biosensor Technology addressing Agricultural Problems

Maria N. Velasco-Garcia; Toby Mottram

Silsoe Research Institute, Wrest Park, Silsoe, Bedford, MK45 4HS, UK; e-mail of Corresponding author: maria.velasco-garcia@bbsrc.ac.uk

(Received 7 June 2001; accepted in revised form 2 October 2002)

Biosensor technology is a powerful alternative to conventional analytical techniques, harnessing the specificity and sensitivity of biological systems in small, low cost devices. Despite the promising biosensors developed in research laboratories, there are not many reports of applications in agricultural monitoring. The authors review biosensor technology and discuss the different bio-receptor systems and methods of transduction. The difference between a biosensor and a truly integrated biosensor system are defined and the main reasons for the slow technology transfer of biosensors to the marketplace are reported. Biosensor research and development has been directed mainly towards health care, environmental applications and the food industry. The most commercially important application is the hand-held glucose meter used by diabetics. The agricultural/veterinary testing market has seen a number of diagnostic tests but no true biosensor systems have made an impact. The need for fast, on-line and accurate sensing opens up opportunities for biosensors in many different agricultural areas —*in situ* analysis of pollutants in crops and soils, detection and identification of infectious diseases in crops and livestock, on-line measurements of important food processing parameters, monitoring animal fertility and screening therapeutic drugs in veterinary testing. Future challenges in the commercial development of biosensor are also addressed.

1. Introduction

A biosensor is described as a compact analytical device, incorporating a biological or biomimetic sensing element, either closely connected to, or integrated within, a transducer system (*Fig. 1*). The principle of detection is the specific binding of the analyte of interest to the complementary biorecognition element immobilised on a suitable support medium. The specific interaction results in a change in one or more physico-chemical properties (pH change, electron transfer, mass change, heat transfer, uptake or release of gases or specific ions) which are detected and may be measured by the transducer. The usual aim is to produce an electronic signal which is proportional in magnitude or frequency to the concentration of a specific analyte or group of analytes, to which the biosensing element binds (Turner *et al.*, 1986; Powner & Yalcinkaya, 1997).

Biosensors can be classified by their bio-recognition system. The main biological materials used in biosensor technology are the couples enzyme/substrate, antibody/antigen and nucleic acids/complementary sequences. In addition, microorganisms, animal or plant whole cells and tissue slices can also be incorporated in the biosensing system. Recent advances and developments in the molecular imprinting area offer an alternative approach involving the use of artificial biomimetic recognition systems. Molecular imprinted polymers can, in principle, be synthesised for any analyte molecule and are capable of binding target molecules with affinities and specifities on a par with biological recognition elements (Haupt & Mosbach, 2000).

Depending on the method of signal transduction, biosensors can also be divided into different groups: electrochemical, optical, thermometric, piezoelectric or magnetic. Amperometric devices are the most commonly reported class of biosensors. Amperometric detection typically relies on an enzyme system that catalytically converts analytes into products that can be oxidised or reduced at a working electrode, maintained at a specific potential. The main advantage of this transducer is the low cost and the use of disposable electrodes. The high reprodu-

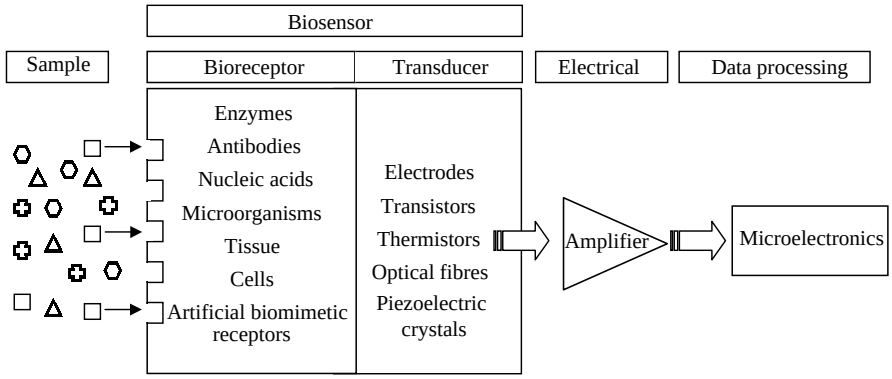

Fig. 1. Principle of operation of a biosensor

cibility of these single use electrodes eliminates the requirement for repeated calibration. Limitations of amperometric transducers include interference from electroactive compounds.

In recent years, a key stimulus for the development of optical biosensors has been the availability of high-quality fibres and optoelectronic components. The optical biosensor format may involve direct detection of the analyte of interest or indirect detection through optically labelled probes and the optical transducer may detect changes in the absorbance, luminiscence, polarisation or refractive index. The advantages of optical biosensors are their speed, the immunity of the signal to electrical or magnetic interference and the potential for higher information content (spectrum of information available) but the main drawback can be the high cost of some instrumentation.

The piezoelectric biosensor is based on measuring frequency changes directly related to mass change on the sensor surface. One of the main advantages of this technique is the real-time binding reaction detection, allowing kinetic evaluation of affinity interactions (feature similar to the surface plasmon resonance biosensors) and, in addition, the low cost of the instrumentation required. Limitations of this transduction method are the need for a calibration of each crystal and the possible variability when coating the surface with the antigen or antibody.

Biosensors offer great advantages over conventional analytical techniques. The selectivity of the biological sensing element offers the opportunity for development of highly specific devices for real-time analysis in complex mixtures, without the need for extensive sample pre-treatment or large sample volumes. Biosensors also promise highly sensitive, rapid, reproducible and simple-to-operate analytical tools.

Despite optimism for the potential of biosensors, their emergence from the research laboratory to the marketplace has been slow. The obstacles to exploitation have been fundamentally related to the presence of biomaterial in the biosensor (immobilisation of biomolecules on transducers, stability of enzymes and antibodies), the development of the sensor device (sensitivity and reproducibility issues) and the integration of biosensors into complete systems. Another major problem for the realistic mass production of biosensors has been the cost factor.

A biosensor system can be defined as the combination of elements such as a method of sampling automatically or manually, a biosensor, a system for replenishing or replacing the biosensor and a data analysis system to implement a biological model which provides information to a human or automated controller. The integration of fluidics, electronics, separation technology and biological subsystems is crucial for the development of biosensor systems. The sensor/sampling system biointerface is a key target for the construction of an integrated system. From the literature, there are many examples of biosensors successfully tested in a laboratory or at prototype level but few examples, even in research, of integrated biosensor systems that offer automatic monitoring in complex matrices.

The potential applications of biosensors in agriculture and food processing are numerous and each has its own requirements in terms of the concentration of analyte to be measured, the required precision of output, the sample volume needed, the time taken to complete the assay, the time necessary for the biosensor to be ready to be used again and the cleaning requirements of the system. The size of the possible market may also have an impact on the type of biosensor specified, as some are more amenable to mass production than others.

This paper summarises the current state of biosensor research applied to agricultural problems. Although most of these devices have not been marketed commercially, they have been successfully tested in the laboratory.

2. Pesticide residues in crop and soil samples

The analysis of pesticide residues is an important concern due to their high toxicity and the serious risk that they represent for the environment and human health. Analysis for pesticides is usually carried out by gas chromatography (GC) or high performance liquid chromatography (HPLC). However, these methods require laborious extraction and clean up steps that increase analysis time and the risk of errors. The development of biosensors is a growing area, in response to the demand for rapid, simple, selective and low cost techniques for pesticides. The main principle of the biosensors developed is based on the correlation between toxicity of a pesticide and a decrease in the activity of a biomarker such as an enzyme. This activity can be registered by employing different transducers, *e.g.* amperometry, potentiometry, spectrometry, fluorimetry or thermometry for detection of different substrates or products of enzymatic reaction.

Organophosphorus and carbamate insecticides selectively inhibit cholinesterases. The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of acetylcholine to acetic acid and choline:

$$(CH_3)_3N(OH)CH_2CH_2OCOCH_3+H_2O \xrightarrow{\text{AChE}} CH_3COOH + (CH_3)_3N(OH)CH_2CH_2OH$$

Several authors have used a pH-sensitive transducer in the development of AchE-based biosensors (Tran-Minh *et al.*, 1990; Andres & Narayanaswamy, 1997), measuring the pH change generated by the release of acetic acid during the enzymatic reaction. Xavier *et al.* (2000) described an optical fibre biosensor for the determination of the pesticides propoxur and carbaryl, widespread insecticides in vegetable crops. The optical transducer was a pH indicator (chlorophenol red) covalently bound to controlled pore glass beads. In the presence of a constant acetylcholine concentration, the colour of the pH-sensitive layer changed proportionally to the carbamate concentration in the sample solution. This colour change varied the reflectance signal (at 602 nm) measured by the fibre optic device. An incubation time of 6 min was chosen and the linear dynamic range for the determination of propoxur and carbaryl was 0·03–3·00 and 0·8–6·0 mg l^{-1}, respectively. The optode produced reproducible (relative standard deviation, RSD 5%) and stable responses for over 4 months. The biosensor was successfully applied to the quantification of propoxur in vegetable samples (onion and lettuce) below the maximum concentrations allowed by the Spanish law (3·0 mg kg^{-1} in fresh or frozen fruits and vegetables). Another example is the multi-enzymatic electrochemical sensor developed by Starodub *et al.* (1999), that was based on a capacitative pH-sensitive electrolyte–insulator–semiconductor (EIS)- structure with silicon nitride ion-sensitive layers as transducers. Determination of heavy metal ions and phosphororganic pesticides in contaminated potatoes and cabbage sap was performed by the sensor array. The multi-enzyme analysis followed by mathematical processing is an effective approach to develop computer-controlled sensor arrays for analysis of toxic substrates.

The amount of choline generated in the enzymatic reaction could also be directly related to the enzyme activity. Nunes *et al.* (1999) used as substrate for the cholisterases enzymes, a salt of acetylthiocholine or butyrylthiocholine (ATCh, BTCh), and the thiocholine produced during the enzymatic reaction was anodically oxidised on a screen-printed electrode. The amperometric biosensor procedure consisted of the deposition of a small drop of substrate or sample (50 µ*l*) on a horizontally positioned biosensor strip representing the microelectrochemical cell. An incubation time of 10 min for enzyme inhibition was needed before addition of the substrate. The biosensors were suitable for single use and no complicated and time-consuming procedures for regeneration of enzyme activity after inhibition were necessary. The linear range of the biosensor for N-methylcarbamates (aldicarb, carbaryl, carbofuran, methomyl and propoxur) varied from 5×10^{-5} to 50 mg kg^{-1}. The cost of carbamate analysis with a biosensor was much lower compared to chromatographic methods, and the analysis was conducted on crop extracts and on vegetable juices at ppb concentration levels without any pre-treatment. By combining native and recombinant variants of acetylcholinesterase with data processing by artificial neural networks, Bachmann *et al.* (2000) reported a multi-analyte detection for organophosphates and carbamates. The assay duration was 40 min and the limit of detection for paraoxon and carbofuran in drinking water in separate analyses was of 0·5 µg ml^{-1}. For inhibition analysis of binary mixtures, the sensor displayed a resolution error of 0·4 µg ml^{-1} for paraoxon and 0·5µg ml^{-1} for carbofuran. The multisensor approach needs improvement as the legal required limits are 0·5 µg ml^{-1} for total and 0·1 µg ml^{-1} for individual pesticide concentration in drinking water (EC Council Directive, 30.08.1980). The hydrophobicity of organophosphorus and carbamic pesticides in water miscible organic solvents was also

evaluated for the development of new biosensors. Palchetti *et al.* (1997) described a choline amperometric biosensor for carbofuran in real samples (fruits and vegetables), using buffers containing 1% v/v acetonitrile.

Biosensors have been reported for pesticides based on the inhibition of acetylcholisterase but using different signal transduction methods. Roda *et al.* (1994) developed a chemiluminiscence-based flow method for the determination of organophorus and carbamate pesticides. The choline formed by the acetylcholisterase was oxidised by choline oxidase and the H_2O_2 produced was via the luminol/peroxidase luminiscent reaction. The detection limits for paraoxon were $125\,\mu g\,l$ and the results obtained were in good agreement with those obtained by a commonly used colorimetric test. Lui *et al.* (1997) developed a biosensor for methamidophos (one of the most commonly used organophosphate insecticides in South East Asia), based on acetylcholinesterase immobilised onto magnetic particles in a photometric flow injection system. The biosensor could detect methamidophos in lettuce and cabbage at 12 and $3\,mg\,kg^{-1}$ vegetable material, respectively. The determination of organophosphate and carbamate pesticides in spiked drinking water and fruit juices was also carried out using a photothermal biosensor (Pogacnik & Francko, 1999). With this approach, $0{\cdot}2\,ng\,ml^{-1}$ of paraoxon was detected in less than 15 min, without any pretreatment step.

In the literature, there are also other biosensors described based on the property of some toxic compounds to reduce the intensity of certain natural processes such as photosynthesis and bioluminescence. Koblizek *et al.* (1998) developed a biosensor for traces of herbicide residues based on a chlorophyll–protein reaction center complex, which measures oxygen levels. If the soil contained a herbicide, the chemical reacted with the biosensor protein and inhibited oxygen production. The biosensor measured herbicides that inhibit photosynthesis, 50% of all herbicides used in agriculture. The test was ultra-sensitive, with detection limits similar to the more complex, highly sensitive enzyme-linked immunosorbent assay (ELISA).

Researchers are starting to address analytical problems as the determination of pesticides using molecular imprinted polymers based biomimetic sensors. Sergeyera *et al.* (1999) reported molecularly imprinted polymer membranes for atrazine as a recognition element of a conductometric sensor. The biosensor developed demonstrated high selectivity and sensitivity (detection limit 5 nM), rapid response (few minutes) and long stability (over 6 months). Also there is reported a rapid, cheap and disposable sensor for 2,4-dichlorophenoxyacetic acid, based on molecular imprinted polymers as recognition elements and electrochemical detection using screen printed electrodes (Kroger *et al.*, 1999).

3. Process control

3.1. *Bacteriological food safety*

Foodborne pathogens cause economic loss, human suffering and even death. Pathogen detection using culture techniques and bioassays such as ELISA for determining and enumerating pathogens in food is well established. However, these methods are very elaborate, very time consuming and cannot be used as on-site monitoring techniques. In recent years, various types of biosensor have been developed which could help in overall quality control in food processing plants by detecting pathogens within minutes of sampling. If pathogens are found with on- or near-line biosensors, then food processors can make decisions more quickly about applying treatments, minimising the chance of a contaminated final product. The general approach for the biosensors described in the literature is an immunoaffinity step to capture and concentrate bacteria on beads, a membrane or a fiber optic probe tip, followed by detection of bound bacteria by laser excitation of bound fluorescent antibodies, acoustogravimetric wave transduction, surface plasmon resonance or electrochemical methods. The main problem facing the production of biosensors for direct detection of bacteria is the sensitivity of the assay in real samples, an issue that still requires improvement. The infectious dosage of pathogens such as *Salmonella* or *Escherichia coli* O157: H7 is 10 cells and the existing coliform standard for *E. coli* in water is $4\,cells\,100\,ml^{-1}$.

Poultry products are presumed to be a major cause of human foodborne illness due to the relatively high frequency of contamination with pathogens *Salmonella* spp. and *Campylobacter* spp. The Threshold Immunoassay System, a biopharmaceutical-contaminant detection system based upon the light-addressable potentiometric sensor (LAPS) has been used to detect *Salmonella* rapidly (in less than 15 min) and reliably, to levels slightly above 100 colony forming units (CFU) (Dill *et al.*, 1999). The immunoassay system utilised solution phase binding of antibodies to *Salmonella*, and the immunocomplex formed is then captured on biotin-coated nitro-cellulose membrane. Finally, a signal generator (an anti-fluorescein urease conjugate) was bound to the immunocomplex. Detection and quantitation of the immunocomplex was made via changes in pH at the silicon chip surface as a result of the conversion of urea to carbon dioxide and ammonia. The limit of detection of the silicon chip-based biosensor is substantially better than the values obtained using enzyme-

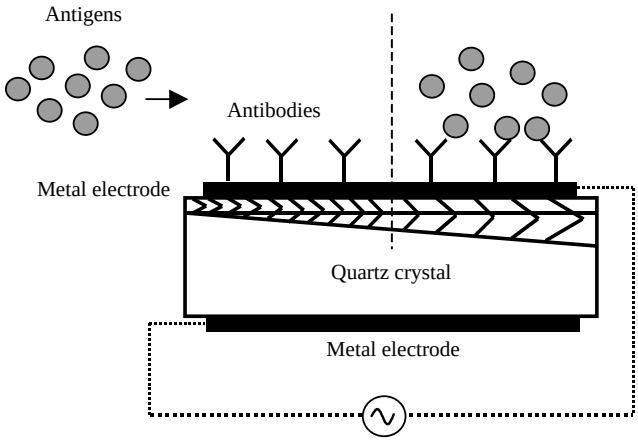

Fig. 2. Piezoelectric crystal biosensor; mass deposition at the surface alters the frequency of the resonant of the crystal

linked methods and comparable with a polymerase chain reaction (PCR)-chemiluminescent method. Experiments involving the detection of salmonella from chicken carcass washing (showing a recovery of 90%) indicated that this technology could be placed into on-site facilities and used to evaluate the extent of salmonella contamination in the poultry industry.

Ye *et al.* (1997) described a piezoelectric biosensor for detection of *Salmonella typhimurium.* The device consists of a quartz crystal wafer sandwiched between two metal electrodes. These electrodes provide a means of connecting the device to an external oscillator circuit that drives the quartz crystal at its resonant frequency. A change in mass on the surface of the electrode thus changes the resonant frequency of the quartz crystal microbalance (QCM) device (*Fig. 2*). The antibody against *Salmonella* was immobilised onto the gold electrode-coated quartz crystal surface through a polyethylenimine–glutaraldehyde technique. The *Salmonella* cells reacted specifically and bound to the crystal surface resulting in an increase in mass that was directly related to the concentration in the solution. The biosensor responded to concentrations of *S. typhimurium* in the range of $5{\cdot}3 \times 10^5$ to $1{\cdot}2 \times 10^9$ CFUml^{-1} in 25 min.

Another biosensor technique for *Salmonella* detection developed by Seo *et al.* (1999) was a direct method, in which *Salmonella* binding to specific antibodies attached to a waveguide surface were detected in minutes by measuring interferometrically the alteration in phase velocity of a guided optical wave. The biosensor was able to detect *S. typhimurium* in chicken carcass wash fluid inoculated at a level of 20 CFU/ml after 12 h of nonselective enrichment. The planar optic biosensor showed promise as a fast, sensitive and economical means of detecting food pathogens.

One of the many applications of surface plasmon resonance (SPR) technology is the detection of *E. coli* O157:H7. Surface plasmon resonance is a quantum optical-electrical phenomenon based on the fact that energy carried by photons of light can be transferred to electrons in a metal. The wavelength of light at which coupling occurs is characteristic of the particular metal and the environment of the metal surface illuminated. This transfer can be observed by measuring the amount of light reflected by the metal surface. *Figure 3* shows how a change in the chemical environment 100 nm above the thin metal layer results in a shift in the wavelength of light, which is absorbed rather than reflected. The most common practical implementation of SPR is to use a metal-coated optical prism, but other practical implementations have been demonstrated including metal-coated diffraction gratings, optical fibres and planar waveguides. The SPR biosensor has potential for use in rapid, real-time detection and identification of bacteria, and to study the interaction of organisms with different antisera or other molecular species (Fratamico *et al.*, 1998). The lower detection limit of the BIAcore system (a commercial example of an SPR system) is approximately 10 pg of analyte mm^{-2}.

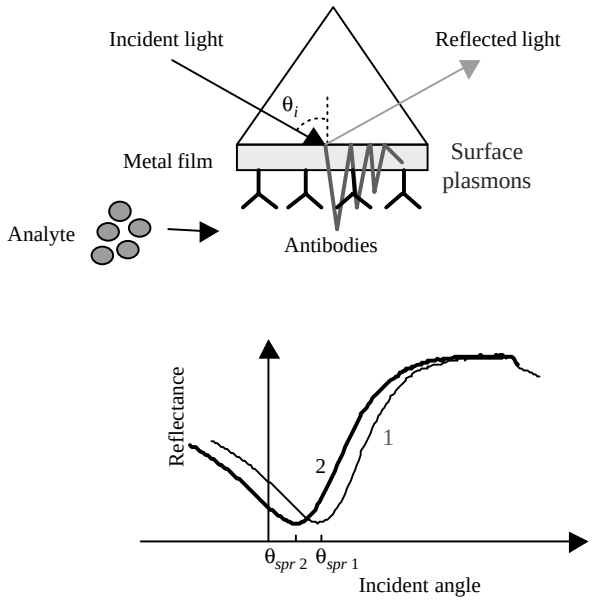

Fig. 3. Surface plasmon resonance (SPR) biosensor; surface plasmons are excited at a specific incident angle (θspr), resulting in a massive reduction in reflectivity at that angle; biomolecular interactions at the metal surface are monitored as a change of the optimal angle required for surface plasmons excitation on the metal layer; SPR curves before (1) and after (2) analyte binding

An evanescent wave biosensor for detection of *Staphylococcal enterotoxin A* in potato salad, milk and mushrooms has also been reported (Rasooly & Rasooly, 1999).

Electrochemical methods coupled with magnetic separation were used to detect *Salmonella* (Che *et al.*, 1999) and *E. coli* (Perez *et al.*, 1998). The methods were completed in less than 2 h and the detection limit was 5×10^3 cell ml^{-1} for the *Salmonella* and 10^5 cell ml^{-1} for the *E. coli*. Recently, there have been important developments in the use of nucleic acid-based assays for detection of foodborne pathogens (Smith *et al.*, 2000; Olsen, 2000). In the future biosensor/deoxyribonucleic acid (DNA)-chip technology could be an alternative to detect microbial diseases.

3.2. *Quality control*

Improper package design or temperature abuse during handling may cause fruits and vegetables in modified-atmosphere packages to be exposed to low, injurious O_2 levels associated with the production of fermentation volatiles, quality loss and eventually product breakdown. Excessively low package O_2 also may promote growth of dangerous pathogens (*e.g. Clostridium botulinum*). The detection of ethanol would provide a sensitive technique for low-O_2 injury identification. A commercial ethanol biosensor composed of a chromagen and immobilised enzymes: alcohol oxidase and peroxidase has been tested. Alcohol oxidase catalyses oxidation of ethanol into acetaldehyde and H_2O_2 in the presence of O_2, and peroxidase (an H_2O_2 decomposing enzyme) catalyses oxidation of the chromagen causing a colour change. The biosensor detected ethanol to levels below the human olfactory threshold [10 $\mu l\, l^{-1}$ ($\approx$ 1 Pa) ethanol in gas phase at 5°C with a 15 s exposure]. The onset of low O_2 injury was detected in lightly processed lettuce, cauliflower, broccoli and cabbage modified-atmosphere packages as measured by accumulation of headspace ethanol (Smyth *et al.,* 1999). The response of the biosensor was very similar to the one measured by gas chromatography, which is expensive and requires technical expertise. The biosensor could also be useful to monitor ethanol during controlled-atmosphere storage of apples, rot development in stored potato tubers or any application where ethanol accumulation can be associated with a loss of quality.

Natural amines in plants are involved in various physiological processes such as the fruit development and senescence. Their levels can vary depending on variety, ripening and storage conditions. Moreover, microbial contamination results in an increase of the biogenic amine content of fruits and vegetables. Electrochemical biosensors for the determination of the amine content in fruits have been assembled using diamine oxidase (DAO) and polyamine oxidase (PAO) covalently immobilised onto polymeric membranes. Both enzymes in the presence of their substrates produced H_2O_2 that was detected at a platinum electrode. The detection limit of DAO and PAO biosensor for the polyamines spermidine and spermine is 10^{-6} mol/l with a good repeatability of 3 RSD%. The linear dynamic range for the analysis of polyamines in real samples was 2×10^{-6}–5×10^{-5} mol/l and the PAO biosensor lifetime was 45 days. The biosensor was applied for determination of amine variations during apricot and sweet cherry ripening under different storage conditions (Esti *et al.*, 1998). Apricots and cherries were stoned and ground to a puree, the homogenate was filtered and the resulting juice was used for the analysis. Biogenic amines (*e.g.* histamine, putrescine, cadaverine, tyramine, cystamine, agmatine, spermide) may also be formed during storage of foodstuffs. Recently, Carsol and Mascini (1999) and Niculescu *et al.* (2000) developed biosensors for monitoring freshness in fish samples, based on electrochemical oxidation of enzymatically produced H_2O_2 in the presence of these chemical indicators.

Biosensing principles, such as the amperometric glucose sensor, have been applied in process control and the food industry. Maines *et al.* (1996) reviewed the requirements for sensing in the food industry and discussed the potential of enzyme electrodes to fulfil the need and the challenges presented by technology transfer into food applications. Examples of successful commercialised sensing instruments are the meatcheck and biocheck sensors. The meatcheck is a four-electrode array attached to a knife, which can be inserted into meat to measure the glucose gradient immediately below the surface. The size of the gradient is related to microbial activity on the surface of the meat and is regarded as a sound indicator of meat quality. The device provides in seconds what laboratory-based microbiology takes days to test. The biocheck method transformed the glucose sensor into a device capable of detecting and quantifying microorganisms in aqueous solutions. The system transferred electrons from the respiratory pathways of micro-organisms, and detected bacteria in under two minutes.

Concentration of lactic acid is an important parameter for the meat industry as it characterises the state of fresh meat. Lactic acid is caused by anaerobic glycolysis from glycogen post mortem in muscles. The lactic acid concentration leads to conclusions concerning the pre-mortem metabolic situation, physical stress and deficiency in the meat quality. Bergann *et al.* (1999)

reported an enzymatic biosensor based on immobilised lactatoxidase as bioreceptor and an amperometric transducer. The biosensor estimates lactic acid without special sample preparation, very quickly and at low cost.

The increasing demand for on-line evaluation of milk quality directs the industry to look for practical solutions, and biosensors are a promising possibility. Eshkenazi *et al.* (2000) developed a multi-enzymatic amperometric biosensor for lactose in fresh raw milk. The characteristics of the biosensor (easy operation, rapid response, long stability) suggested that this method could be used as an economical on-line lactose measurement technique in the milking parlour. Also in the literature there are biosensors described for fat in milk. Schmidt *et al.* (1996) reported a microbial biosensor based on thick film technology for free fatty acids. The biosensor measured the oxygen uptaken by respiratory activity of the immobilised microorganisms. Oxygen was determined by electrochemical reduction. The sensor could be applied to milk samples without previous pretreatment, having a short response, high sensitivity and easy handling. However, biological research is needed to determine how sensor derived information can be used to improve the product quality other than by separating the milk into sources of high and low quality.

4. Biosensors for detection and identification of infectious disease in crops

Some microorganisms, particularly certain bacteria and fungi, are pathogens that attack crops and cause disease, sometimes in epidemic proportions. Fungal infection and aflatoxin production can occur at any stage of plant growth, harvesting, drying, processing and storage. Exposure by ingestion or inhalation of aflatoxins may lead to the development of serious medical conditions (structural and functional damage of the liver, hepatic encephalopathy, immunosuppression, lower respiratory infections, gastrointestinal haemorrhage, anorexia, malaise, fever). US government agencies monitor and tightly regulate aflatoxin levels in animal feed and various food products. Action levels above which human food products are removed from commerce are typically 20 ppb.

Recently an immuno-affinity fluorimetric biosensor was developed for detecting and quantifying aflatoxins, a group of chemically related mycotoxins formed by common fungi (*Aspergillus flavus, A. parasiticus* and *A. nomius*) found in maize, cottonseed, peanuts, and other nuts, grains and spices. The sample was filtered through a column containing sepharose beads to which the polyclonal aflatoxin-specific antibodies were attached. The beads with the attached bound aflatoxin were subsequently rinsed to remove any unbound or non-specifically bound impurities and interferents. After the rinse, an eluant solution was passed through the beads causing the antibodies to release the bound aflatoxin. The analyte was then collected and placed in a fluorimeter. There were essentially two subsystems within this biosensor: a fluidics subsystem, which performed mechanical sample-handling and processing, and an electro-optical system incorporating a miniature fluorometer that measured and reported the toxin level to the user. The two systems were controlled by a microprocessor that directed the fluidics and electro-optical system to perform the analysis. The aflatoxin biosensor provided a portable multisample, rapid sampling and measurement capability with minimal sample handling and consumables. It provided very high sensitivity from 0·1 to 50 ppb, in less than 2 min with a 1 m*l* sample and made over 100 measurements before refurbishment was required (Carlson *et al.*, 2000).

Schutz *et al.* (2000) developed a biosensor to detect marker volatiles released by potato tubers infested with *Phytophtora infestans*, offering a possible solution to the problem of screening large numbers of seed potatoes for fungal infestation. The biosensor, based on the intact antennae of Colorado potato beetle (*Leptinotarsa decemlineata*), was able to detect one single diseased potato tuber within up to 100 kg potato tubers, offering a promising early warning technique. Insect antennae are very suitable for the construction of highly sensitive biosensor, because of their remarkable sensory abilities.

5. Animal production

5.1. *Oestrus detection*

In the cattle breeding industry, where artificial insemination techniques are employed, the successful prediction of oestrus onset leads to considerable cost saving in herd management. Visual observation is the most commonly used way of detecting oestrus, however, oestrus does not always accompany ovulation and can occur in pregnant animals. Monitoring progesterone levels in milk is a more effective method not only of predicting ovulation but also for detecting pregnancy and fertility problems. The optimum fertility rates are achieved when insemination is performed 3 days after the progesterone level falls to below 5 ng ml^{-1} of whole milk. ELISA test kits have allowed oestrus detection with 98% specificity but these methods require time and skills not abundant on a typical farm. Development of a

real-time milk progesterone biosensor would provide a very useful tool for fertility monitoring.

Several approaches have been developed by researchers to determine progesterone concentration in milk. Pemberton *et al.* (1998) reported a disposable screen-printed amperometric progesterone biosensor, operated in a competitive immunoassay format. The biosensor relies upon a reduction in the binding to the sensor surface of alkaline phosphatase-labelled progesterone in the presence of endogenous milk progesterone. The enzyme substrate is napthyl phosphate. The 1-napthol generated in the reaction is electrochemically oxidised, producing a signal inversely proportional to the concentration of unlabelled progesterone in milk. By using screen-printing technology, it is possible to mass-produce the transducer element and fabricate sensors at low cost, enabling the screen-printed electrodes to be disposable devices. Mottram *et al.* (2000a) are transferring this technology, developing an automated ovulation system for progesterone in whole fresh milk, linked to a herd management database. The prototype biosensing system detects concentrations of progesterone between 3 and 30 ng ml^{-1} in whole fresh milk (normal physiological levels), with a good regression (coefficient of determination $R^2 = 0{\cdot}965$).

Another biosensor for oestrus detection, monitoring on-line bovine progesterone during milking, employed an enzyme immunoassay format for molecular recognition but in this case the transducer was optical (Claycomb *et al.*, 1998). The endogenous progesterone present in milk competed with progesterone labelled with horseradish peroxidase (HRP) for binding the active biosensing surface (a nitrocellulose membrane). The substrate was a solution of 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2 and the transduction was based on the oxidation of TMB, generating a blue product. A spectrophotometer read a signal at 650 nm that is inversely proportional to the concentration of progesterone in milk.

5.2. *Veterinary drug residue screening*

The use of antibiotics and chemotherapeutics in animal husbandry has led to the occurrence of veterinary drug residues in foods of animal origin. Traditional microbial screening methods have insufficient sensitivities to meet new regulations and classical physiochemical techniques, such as chromatographic methods and mass spectrometry are often precluded due to the level of experience, skills and cost involved. Immunological techniques have become increasingly popular for monitoring levels of therapeutic substances.

Sulphonamides are a family of chemotherapeutics that are widely used for therapeutic and prophylactic purposes in animal diseases. In the treatment of mastitis, sulphonamides are usually administrated in the case of infections caused by Gram-negative pathogens, *e.g. E. coli*. Toxicological data show that sulphonamides have antithyroid effects in both animals and humans. In Europe, a maximum residue limit of 0·1 mg kg^{-1} has been established for total sulphonamides in milk. A surface plasmon resonance biosensor was compared with existing methods (microbial inhibitor assays, microbial receptor assays, ELISA, HPLC) for detection of sulfamethazine (SMZ) residues in milk (Mellgren *et al.*, 1996). The Pharmacia BIAcore (a commercial surface plasmon resonance system) indicated the occurrence of less than 0·9 μg of SMZ per kg of milk (concentration below the detection limit of HPLC) and offered sufficient advantages (no sample preparation, high sensitivity rapid and fully analysis in real time) to be an alternative for the control of residues and contaminants in food. Baxter *et al.* (1999) reported the first study conducted to determine the feasibility of performing on-site drug screening at an abattoir, using an immunobiosensor. The biosensor was used for screening for SMZ in pig bile samples, using a predetermined threshold limit of 0·4 μg m*l-1*. This method gave an accurate indication that the corresponding tissue sample contained SMZ residues in excess of the maximum residue limit. All positive control pigs were correctly identified during testing. A false positive rate of 0·3% was obtained but no false negatives were generated.

Enrofloxacin is a synthetic antimicrobial agent of the fluoroquinolone family, especially designed for use in veterinary medicine (to treat mastitis). In the cow, enrofloxacin has a long elimination time in milk. In general, microbiological inhibitor assays for routine control of inhibitory substances in milk fail to detect fluoroquinolone residues at low levels. A rapid, sensitive surface plasmon resonance biosensor for enrofloxacin and its main metabolite, ciprofloxacin, in milk was developed (Mellgren & Sternesjo, 1998).

The drug salbutamol (SBL) is a beta-agonist that may be used illegally as an animal growth promoter. Elliot *et al.* in 1998 using the commercially available surface plasmon resonance biosensor instrument, (Biacore, Uppsala, Sweden) analysed salbutamol (SBL) in urine samples of calves treated with SBL orally for 3 days. This work showed that biosensor-based veterinary drug residue testing procedures can generate results in real time without the need for time-consuming sample preparation.

Setford *et al.* (1999) developed a screen-printed device to measure penicillin G levels in milk. The biosensor was

based on an ELISA affinity assay coupled to amperometric determination of bound enzyme label activity. The system is ideal as a field-based screening tool for beta-lactam quantification in milk. More recently Delwiche *et al.* (2000) reported an enzyme immunoassay for beta-lactam penicillins, but in this case the system used a photometric sensor as transducer.

5.3. *Veterinary diagnosis*

Biosensors are a promising tool to diagnose and thereby aid in controlling animal diseases, but there are very few examples of biosensors applied to veterinary diagnosis and they are mainly piezoelectric immunosensors.

Wu *et al.* (1997) reported a liquid piezoelectric immunoassay to determine the infection of rabbit serum by adult worm antigen of *S. japonicum*. In 1998 a direct piezoelectric flow injection analysis (FIA) immunoassay was developed for the detection of African Swine Fever virus (Uttenthaler *et al.*, 1998). The calibration curves in buffer and in serum could be determined and proved the suitability of the quartz crystal biosensor for the classification of positive and negative pig serum samples. More recently, Su *et al.* (2000) reported a piezoelectric immunosensor for porcine reproductive and respiratory syndrome virus (PRRSV). The proposed biosensor was used to screen pigs suspected to have been infected with the virus and to provide positive or negative results in a few minutes. Kumar (2000) developed a method for diagnosis of tuberculosis and other infections caused by mycobacteria. The preliminary results presented with the piezoelectric immunosensor for mycobacterial antigen (in gas and liquid phase), offer an enormous potential. For instance, detection of the antigen in saliva could constitute a non-invasive method of screening high-risk population. The potential could then be demonstrated for animal disease exposures to be detected without the need for blood sampling and off-line laboratory analysis. Biosensor systems to detect infectious diseases at ports and in field situations without the need for expensive veterinary support would be a major asset for monitoring and controlling animal diseases.

Biosensor technology could also be applied to detect mastitis infection by sensing markers such as enzyme *N*-acetylglucosaminidase (NAGase) in milk. This enzyme is released into milk as a result of tissue damage when the cow is resisting a clinical intra-mammary infection. Mottram *et al.* (2000b) showed the potential for a sensor based on the ability to convert 1-naphthyl *N*-acetyl-*b*-*D*-glucosaminidine to 1-naphthol, which can be detected electrochemically.

6. Future challenges

Application and commercialisation of biosensor technology has lagged behind the output of research laboratories. Although many biosensor-related patents are filed each year, very few play a prominent role in clinical diagnostic, food industry, environmental agricultural or veterinary applications. There have been many reasons for the slow technology transfer from the research laboratories to the marketplace: cost considerations, stability and sensitivity issues, quality assurance, instrumentation design. Many of the main barriers are technical, methods of sensor calibration, methods of producing inexpensive and reliable sensors, stabilisation and storage of biosensors, and total integration of the sensor system.

The behaviour of biomolecules adsorbed and immobilised on surfaces during the fabrication and use of biosensors needs to be better understood. In recent years, there has been remarkable progress in surface chemical analysis and scanning probe microscope techniques such as atomic force microscopy (AFM) and scanning tunnelling microscopy (STM) but unfortunately our knowledge of manipulation and improvement of protein stability is far from complete. The limited stability of proteins (enzymes, antibodies) acts as a brake on the development of biosensors.

One of the future challenges is to develop cost-effective methods for sequencing, interpreting and storing deoxyribonucleic acid (DNA) sequences. The development of DNA probes is a promising area of research in biosensors. Deoxyribonucleic acid sensors could be used to detect polymorphism or mutations in genes within plants, animals and microorganisms.

Biomimetic systems will also accelerate biosensor development and applications. Some researchers are trying to overcome the poor stability of biological molecules by developing artificial molecular recognition systems with predetermined selectivity for various substances. So far, molecular imprinted polymers have been prepared with affinities for proteins, aminoacid derivatives, sugars, vitamins, pesticides, and pharmaceuticals. Some advantages of molecular imprinting *versus* biological receptors are the low cost, ease of synthesis and relative long-term stability.

The development of automated manufacturing technologies is extremely important in the commercial mass production of biosensors. Deposition techniques such as screen-printing and ink-jet printing allow printing of materials at very high precision and speed, producing large numbers of inexpensive and reproducible biosensors. Thin-film deposition techniques such as Langmuir-Blodgett technology, are able to create layers less than 1 μm thick, suitable for the development of

microsensors. Another area of intense research activity is silicon fabrication technology. The idea of lab-on-a-chip is very attractive. The fabrication of credit-card sized microlaboratories will rely on advanced micro-fabrication and micromachining technologies. It is now technically feasible to miniaturise and integrate complex components such as pumps, valves, mixers and flow cells in a single chip along with the biosensor.

Biosensor advancement in the commercial world could also be accelerated by the use of intelligent instrumentation, electronics, and multivariate signal-processing methods such as chemometrics and artificial neural networks. Increasing attention will have to be paid to the engineering of both the basic components and the device as a whole. It is in this area where agricultural engineers will have a key role in applying their knowledge of systems to improve sampling, calibration and data analysis to provide instructions for a farmer or processor rather than raw data. A biosensor array strategy, adaptable to multiple analytes detection, will allow spreading development costs over several products. These future improvements will produce devices more competitive with the presently available instruments, and be able to operate under field conditions.

7. Conclusions

The application of biotechnology would seem to be vital in maintaining the productivity and health of crops and livestock in the face of environmental concerns, limited resources and population increases.

Biosensors could play an important role in providing powerful analytical tools to the agricultural diagnosis sector, particularly where rapid, low cost, high sensitivity and specificity measurements in field situations are required. This review summarised on-going developments in this field.

A range of molecules with biorecognition properties can be used as the sensing element in biosensors. A wide range of transducers is also available to engineer new biosensing devices. There are many different ways to combine biology, chemistry, physiscs, mathematics and engineering in order to develop new biosensors with applications in agriculture.

The promise shown by biosensor technology is very real, however there are some technological obstacles that need to be overcome. Advances in areas such as surface chemical analysis, protein stabilisation, automated manufacturing technologies would widen the market and allow biosensors to be more competitive in the agricultural market.

Acknowledgements

This work was funded as part of a BBSRC Central Strategic Grant.

References

Andres R T; Narayanaswamy R (1997). Fibre-optic pesticide biosensor based on covalently immobilized acetylcholinesterase and thymol blue. Talanta, **44,** 1335–1352

Bachmann T; Leca B; Vilatte F; Marty J L; Fournier D; Schmid R D (2000). Improved multianalyte detection of organophosphates and carbamates with disposable multi-electrode biosensors using recombinant mutants of Drosophila acetylcholinesterase and artificial neural networks. Biosensors & Bioelectronics, **15,** 193–201

Baxter G A; Oconnor M C; Haughey S A; Crooks S R H; Elliott C T (1999). Evaluation of an immunobiosensor for the on-site testing of veterinary drug residues at an abattoir. Screening for sulfamethazine in pigs. Analyst, **124** (9), 1315–1318

Bergann T; Gifley K; Abel P (1999). Concentration of lactic acid in carcasses and fresh meat-estimation with an enzymatic-biosensor measuring system. Fleischwirtschaff, **79** (1), 84–87

Carlson M A; Bargeron C B; Benson D C; Fraser A B; Phillips T E; Velky J T; Groopman J D; Strickland P T; Ko H W (2000). An automated, handheld biosensor for aflatoxin. Biosensors & Bioelectronics, **14,** 841–848

Carsol M A; Mascini M (1999). Diamine oxidase and putrescine oxidase immobilized reactors in flow injection analysis: a comparison in substrate specificity. Talanta, **50** (1), 141–148

Claycomb R W; Delwiche M J; Munro C J; BonDurant R H (1998). Rapid enzyme immunoassay of bovine progesterone. Biosensors & Bioelectronics, **13** (11), 1165–1171

Che Y H; Yang Z P; Li Y B; Paul D; Slavik M (1999). Rapid detection of *Salmonella typhimurium* using an immunoelectrochemical method coupled with immunomagnetic separation. Journal of Rapid Methods and Automation in Microbiology, **7** (1), 47–59

Delwiche M; Cox E; Goddeeris B; Van Dorpe C; De Baerdemaeker J; Decuypere E; Sansen W (2000). A biosensor to detect penicillin residues in food. Transactions of the ASAE, **43** (1), 153–159

Dill K; Stanker L H; Young C R (1999). Detection of salmonella in poultry using a silicon chip-based biosensor. Journal of Biochemical and Biophysical methods, **41**, 61-67

Elliot C T; Baxter G A; Hewitt S A; Arts C J M; VanBaak M; Hellenas K E; Johannson A (1998). Use of biosensors for rapid drug residue analysis without sample deconjugation or clean up: a possible way forward. Analyst, **123** (12), 2469–2473

Eshkenazi I; Maltz E; Zion B; Rishpon J (2000). A three-cascaded-enzymes biosensor to determine lactose concentration in raw milk. Journal of Dairy Science, **83**(9), 1939–1945

Esti M; Volpe G; Massignan L; Compagnone D; La Notte E; Palleschi G (1998). Determination of amines in fresh and modified atmosphere packaged fruits using electrochemical biosensors. Journal of Agricultural and Food Chemistry, **46**(10), 4233–4237

Fratamico P M; Strobaugh T P; Medina M B; Gehring A G (1998). Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor. Biotechnology Techniques, **12** (7), 571–576

Haupt K; Mosbach K (2000). Molecularly imprinted polymers and their use in biomimetic sensors. Chemical Reviews, **100**(7), 2495–2504

Koblizek M; Masojidek J; Komenda J; Kucera T; Pilloton R; Mattoo A K; Giardi M T (1998). A sensitive photosystem II-based biosensor for detection of a class of herbicides. Biotechnology and Bioengineering, **60** (6), 664–669

Kroger S; Turner A P F; Mosbach K; Haupt K (1999). Imprinted polymer based sensor system for herbicides using differential pulse voltammetry on screen-printed electrodes. Analytical Chemistry, **71** (17), 3698–3702

Kumar A (2000). Biosensors based on piezoelectric crystal detectors. *http://www.tms.org/pubs/journals/JOM/0010/Kumar/Kumar-0010.htmc*

Lui J; Gunther A; Bilitewski U (1997). Detection of methamidophos in vegetables using a photometric flow injection system. Environmental Monitoring and Assessment, **44** (1–3), 375–382

Maines A; Ashworth D; Vadgama P (1996). Enzyme electrodes for food analysis. Food Technology and Biotechnology, **34**(1), 31–42

Mellgren C; Sternesjo A; Hammer P; Suhren G; Bjorck L; Heeschen W (1996). Comparison of biosensor, microbiological, immunochemical and physical methods for detection of sulfamethazine residues in raw milk. Journal of Food Protection, **59** (11), 1223–1226

Mellgren C; Sternesjo A (1998). Optical immunobiosensor assay for determining enrofloxacin and ciprofloxacin in bovine milk. Journal of AOAC International, **81** (2), 394–397

Mottram T; Hart J; Pemberton R (2000a). A sensor based automatic ovulation prediction system for dairy cows. Proceedings of 5th AISEM Conference. Lecce, Italy

Mottram T; Hart J; Pemberton R (2000b). Biosensing techniques for detecting abnormal and contaminated milk. Robotic Milking. Proceedings of the International Symposium. The Netherlands

Niculescu M; Nistor C; Frebort I; Pec P; Mattiasson B; Csoregi E (2000). Redox hidrogel-based amperometric bienzyme electrodes for fish freshness monitoring. Analytical Chemistry, **72** (7), 1591–1597

Nunes G S; Barcelo D; Grabaric B S; Diaz Cruz J M; Ribeiro M L (1999). Evaluation of a highly sensitive amperometric biosensor with low cholinesterase charge immobilized on a chemically modified carbon paste electrode for trace determination of carbamates in fruit, vegetable and water samples. Analytica Chimica Acta, **399**(1–2), 37–49

Olsen J E (2000). DNA-based methods for detection of food-borne bacterial pathogens. Food Research International, **33** (3–4), 257–266

Palchetti I; Cagnini A; DelCarlo M; Coppi C; Mascini M; Turner A P F (1997). Determination of anticholinesterase pesticides in real samples using a disposable biosensor. Analytica Chimica Acta, **337** (3), 315–321

Pemberton R M; Hart J P; Foulkes J A (1998). Development of a sensitive, selective, electrochemical immunoassay for progesterone in cow's milk based on a disposable screen-printed amperometric biosensor. Electrochimica Acta, **43** (23), 3567–3574

Perez F G; Mascini M; Tothill I E; Turner A P F (1998). Immunomagnetic separation with mediated flow injection analysis amperometric detection of viable *Escherichia coli* O157. Analytical Chemistry, **70** (11), 2380–2386

Pogacnik L; Franko M (1999). Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection. Biosensors & Bioelectronics, **14** (6), 569–578

Powner E T; Yalcinkaya F (1997). Intelligent biosensors. Sensor Review, **17** (2), 107–116

Rasooly L; Rasooly A (1999). Real time biosensor analysis of Staphylococcal enterotoxin A in food. International Journal of Food Microbiology, **49** (3), 119–127

Roda A; Rauch P; Ferri E; Girotti S; Ghini S; Carrea G; Bovara R (1994). Chemiluminiscent flow sensor for the determination of paraoxon and aldicarb pesticides. Analytica Chimica Acta, **294** (1), 35–42

Schutz S; Weissbecker B; Koch U T; Hummel H E (2000). Detection of volatiles released by diseased potatoe tubers using a biosensor on the basis of intact insect antennae. Biosensors & Bioelectronics, **14** (2), 221–228

Seo K H; Brackett R G; Hartman N F; Campbell D P (1999). Development of a rapid response biosensor for detection of *Salmonella Typhimurium*. Journal of Food Protection, **62**(5), 431–437

Sergeyera T A; Piletsky S A; Brovko A A; Slinchenko E A; Sergeeva L M; El'skaya A V (1999). Selective recognition of atrazine by molecularly imprinted polymer membranes. Development of conductimetric sensor for herbicides detection. Analytica Chimica Acta, **392** (2–3), 105–111

Setford S J; Van Es R M; Blankwater Y J; Kroger S (1999). Receptor binding protein amperometric affinity sensor for rapid beta-lactam quantification in milk. Analytica Chimica Acta, **398** (1), 13–22

Schmidt A; Standfuβ-Gabisch C; Bilitewski U (1996). Microbial biosensor for free fatty acids using an oxygen electrode based on thick film technology. Biosensors & Bioelectronics, **11** (11), 1139–1145

Smith T J; O'Connor L; Glennon M; Maher M (2000). Molecular diagnostics in food safety: rapid detection of food-borne pathogens. Irish Journal of Agricultural and Food Research, **39** (2), 309–319

Smyth A B; Talasila P C; Cameron A C (1999). An ethanol biosensor can detect low-oxygen injury in modified atmosphere packages of fresh-cut produce. Postharvest Biology and Technology, **15**(2), 127–134

Starodub N F; Kanjuk N I; Kukla A L; Shirshov Y M (1999). Multi-enzymatic electrochemical sensor: field measurements and their optimisation. Analytica Chimica Acta, **385** (1-3), 461–466

Su X D; Li S F Y; Kwang J; Low S (2000). Piezoelectric quartz crystal based screening test for porcine reproductive and respiratory syndrome virus infection in pigs. Analyst, **125** (4), 725–730

Tran-Minh C; Pandey P C; Kumaran S (1990). Studies on acetylcholine sensor and its analytical applications based on the inhibition of cholinesterase. Biosensors & Bioelectronics, **5,** 461-471

Turner A P F; Karube I; Wilson S W (1986). Biosensors. Fundamentals and Applications. Oxford Science Publications, Oxford

Uttenthaler E; Kosslinger C; Drost S (1998). Quartz crystal biosensor for detection of African Swine Fever disease. Analytica Chimica Acta, **362** (1), 91–100

Wu Z Y; Shen G L; Yu R Q; Wang S P; Zeng X F (1997). Piezoelectric immunosensor based on the agglutination of PEG for determination of S-japonicum antibody. Chemical Journal of Chinese Universities, **18** (11), 1774–1778

Xavier M P; Vallejo B; Marazuela M D; Moreno Bondi M C; Baldini F; Falai A (2000). Fiber optic monitoring of carbamate pesticides using porous glass with covalently bound chlorophenol red. Biosensors & Bioelectronics, **14** (2), 895–905

Ye J; Letcher S V; Rand A G (1977). Piezoelectric biosensor for detection of *Salmonella typhimurium*. Journal of Food Science, **62** (5), 1067–1071